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Anatomical and Physiological Changes in the Pituitary Glands of Vitamin A Deficient Rats

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ANATOMICAL AND PHYSIOLOGICAL CHANGES IN THE PITUITARY GLANDS OF VITAMIN A DEFICIENT RATS

T. S. SUTTON AND B. J. BRIEF[1]

INTRODUCTION

The failure of reproduction among laboratory animals on diets deficient in vitamin A has long been established. Recent investigations indicate that a deficiency of vitamin A is one of the factors contributing to reproductive disorders among farm animals maintained for extended periods of time on poor quality rations. The physical symptoms presented by farm animals suffering from this deficiency are extremely variable and frequently lead to confusion in making an accurate diagnosis. One of the prerequisites to an accurate interpretation of symptoms and the correct diagnosis of any disease is a thorough understanding of the mode of action of the pathogen or, as in this case, the nutritional deficiency, in producing the disease.

It has been established that one of the pathological changes found with striking regularity in avitaminosis-A is a degeneration of the germinal epithelium of the gonads, particularly in the male. The reciprocal relationship known to exist between the gonad and the pituitary gland leads to the assumption that the deficiency may act directly in causing damage to the gonads themselves or indirectly through an endocrine relationship in which the gonad becomes nonfunctional and undergoes atrophic changes because of a hypofunction of the endocrine gland (the pituitary) from which its stimulating principle emanates.

In this study, the authors are concerned with the way vitamin A deficiency works to produce the pathological and physiological changes coincident with sterility in both sexes.

REVIEW OF THE LITERATURE

Numerous investigations, beginning with those of Smith (25), have shown a relationship, reciprocal in nature, between the gonad and the pituitary. The interdependence of these glands for the humoral stimuli regulating their functional activity is well established.

Distortion of the endocrine relationship resulting in sterility has been reported in dietary deficiencies. Parks (23) and Marrian and Parks (15) noted that females on a diet deficient in vitamin B became anoestrus. An assay of the pituitary glands of these anoestrus animals showed them to contain less gonad-stimulating hormone than those from normal controls. Treatment with anterior pituitary substance resulted in immediate ovarian stimulation. Evans and Simpson (6) reported that the hypophyses of vitamin B deficient males were markedly lower in gonad-stimulating power than those of litter mate controls or younger animals of the same body weight. Orten and Smith (22) have shown that a diet low in protein produces similar results. In their study, Orten and Smith also produced further evidence that the impaired activity of the

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reproductive organs was a result of pituitary hypofunction rather than of a direct dietary injury to the gonad. Treatment with the gonadotropic principle of pregnant mare's serum gave a response comparable to that obtained in similarly treated immature females.

The data at hand provide evidence that the reproductive disorders of vitamin E deficiency are caused by direct injury to the reproductive organs. Van Wagenen (30) reported an increase in size and number of basophilic cells in the hypophyses of vitamin E deficient male rats. These rats also showed degeneration of the germinal epithelium. These findings were substantiated by Nelson (20), who also provided evidence that the hypophyses of vitamin E deficient male rats are more potent for gonadotropic hormone (or hormones) than those of normal rats but less potent than those of castrates. On the other hand, females on the vitamin E deficient diet had normal hypophyses when measured histologically and physiologically. This condition in the vitamin E deficient female was corroborated by Stein (27).

Mason and Wolfe (17) have produced physiological evidence that vitamin A deficiency has a direct damaging effect on the reproductive organs. They compared the gonad-stimulating capacities of the hypophyses of castrate and noncastrate vitamin A deficient animals with those of litter mate castrate and noncastrate animals on an adequate diet. The ovaries of the test animals which received hypophyseal transplants from vitamin A deficient noncastrates were 43 per cent greater in weight than those of the test animals that received transplants from normal stock controls. The ovaries of the test animals receiving transplants from vitamin A deficient castrates were 39 per cent greater in weight than those of test animals receiving transplants from vitamin A deficient noncastrates and 100 per cent greater than those of test animals receiving transplants from normal controls. It would appear that the increase in gonad-stimulating properties of the pituitaries represents an attempt on the part of the pituitary to compensate for the direct damage of the dietary regime on the gonad.

Such a marked change in the physiological activity of the pituitary glands should be accompanied by a structural or cytological change in the pituitary of sufficient magnitude to be measured quantitatively.

The classical work of Wolbach and Howe (32) in which they made an exhaustive study of the pathology of vitamin A deficiency disease reported a shrinkage of the gland cells of the pituitary but "no change in the architecture of the gland." Thatcher and Sure (29) reported no changes in the hypophysis in vitamin A deficiency. It is quite possible that these investigators were looking for epithelial metaplasia in the pituitary, which apparently does not occur in vitamin A deficiency.

Mitzkewitsch (19) reported a hypertrophy of the pituitary without histological change in vitamin A deficient rats. It should be noted, however, that this hypertrophy was relative to body weight. The pituitaries of the vitamin A deficient animals were actually much smaller than those from the normal controls. Korenchevsky (13) reported an increase in number and size of the "light cells" of the hypophyses of pigeons in vitamin A deficiency. It is quite probable that both chromophobe and basophilic cells are included in the "light cell" classification. A recent report by Frank (9) states that no macroscopic nor microscopic changes occur in the hypophyses of rats fed on a diet deficient in vitamin A. In reviewing the work in this field the scarcity of data becomes very evident.

Cellular changes in the hypophyses of castrate rats have been studied by several workers, notably Addison (1), Nukariya (21), and Ellison and Wolfe (3). These workers are in agreement that there is a progressive increase in the basophilic elements following castration and that the maximum is reached about 30 days after castration.

Wolfe and Cleveland (33) have shown that there are cyclic changes in the cellular elements of the hypophyses of normal females. These changes are qualitative rather than quantitative. Differences in the actual percentages of alpha, beta, and chromophobe cells at the various stages of the cycle are insignificant.

ANATOMICAL CHANGES

MATERIALS AND METHODS

In this work, 24-day-old weanlings of a closely inbred strain of *Mus norvegicus albinus* were depleted of vitamin A according to the method of U. S. P. XI. After depletion, as indicated by cessation of growth, the diet was supplemented with standard reference cod-liver oil varying in amount from 1 to 1.4 U. S. P. vitamin A units per day for the duration of the experiment. The period between weaning and depletion was usually about 5 to 6 weeks. The animals were maintained on the vitamin A supplemented diet for approximately 7 weeks. These rats, 108 to 112 days old at the termination of the experiment, showed symptoms of extreme vitamin A deficiency by loss of weight, xerophthalmia, muscular incoordination, and respiratory disorders.

The animals used as normal controls were maintained on a complete diet and sacrificed at 95 to 113 days of age. No attempt was made to sacrifice all the normal females at the same stage of the oestrus cycle, since Wolfe and Cleveland (33) have shown that quantitative cyclic variations in the cellular elements of the hypophysis are insignificant. The castrates were operated upon at 50 to 56 days of age just 30 days before termination of the experiment.

All animals were sacrificed by exsanguination after preliminary ether anaesthetization.

The pituitaries of all animals were treated as nearly alike as possible. After fixation in Zenker-formol solution, the glands were embedded in paraffin and cut at 5 micra. The sections were in the horizontal plane of the gland as it lies in the body.

The staining technique was that of Masson as described by Foot (8), in which ponceau de xylidine, acid fuchsin, and aniline blue are used as cytoplasmic stains.

When the stained sections were examined it was apparent that certain regions of the anterior lobe contained concentrations of cells of one particular type. These areas were found to be relatively constant in position in the different glands. It was, therefore, decided to count all the cells on a line through the greatest diameter of the section at a tangent to the innermost projection of the posterior lobe. Although this method does not give a true percentile count of all the cells of the anterior hypophysis, it does permit the rapid counting of all the cells on one axis of the gland. By counting the cells along similar axes in other glands at the same level, comparisons can be made between the percentages of the various cell types present.

In practice the technique involves the use of cross hairs in the microscope ocular and a mechanical stage. The one hair is superimposed on the gland so as to lie upon the imaginary axis described. As the slide is moved by means of the mechanical stage in a direction parallel to that of this hair, a procession of cells crosses a second hair which is at a right angle to the first. As the cells pass the intersection of the cross hairs they are counted differentially. This method leaves less to the judgment of the observer than does the count of whole fields.

Three different sections from the middle of each gland were counted. The counts were made at a magnification of 970×, and an oil immersion objective was used.

The gonads of both sexes and the uteri and vaginae of the females were sectioned, stained with H and E, and examined. This examination was undertaken in order to verify the gonadal changes which are described in the literature as being associated with vitamin A deficiency and also to determine the extent of such changes.

OBSERVATIONS AND DISCUSSION

The cells of the anterior lobe of the hypophysis were classified, for the purposes of this experiment, into three large groups. Those cells which stained with acid fuchsin revealing either a granular or an amorphous cytoplasm were designated as alpha cells. These correspond to the "eosinophiles" or "acidophiles" of the literature. The beta cells were in general larger than the alpha cells and their cytoplasmic granules stained with aniline blue. These are the "basophiles" of the literature. The chromophobes are small cells stained poorly by ponceau de xylidine.

The results of the counts are presented in table 1 and summarized in table 2.

It should be emphasized that the large percentile increase in the number of beta cells in the vitamin A deficient animals does not reflect an equivalent increase in their absolute number, since there is some atrophy of the gland. There was, however, an increase in absolute number of beta cells showing a macula. The increase in number of true castration (signet-ring) cells was insignificant.

There was a significant increase in the number of beta cells in the hypophyses of the vitamin A deficient members of both sexes. This increase approached the condition found in the true castrates used in this study. Reference to table 2, however, shows a relatively greater increase in beta cells in the vitamin A deficient male over the normal male than in the vitamin A deficient female over the normal female. The greater increase may be accounted for on the basis of a more nearly complete destruction of the germinal epithelium of the male gonad. In the male there was an almost complete disappearance of the germinal epithelium, although occasional spermatogonia were to be found in all the testes. No gametes were seen which had matured beyond the primary spermatocyte stage although aberrant forms arising from abortive attempts at spermatogenesis were common. The basement membrane of the tubules remained, as did the Sertoli cells and the interstitial tissue. These tissues became more prominent with the loss of the epithelium but gave no evidence of actual hypertrophy. Figures 1 and 2 are typical illustrations of the histological appearance of testes from normal and vitamin A deficient males. Figure 3 is a greater magnification of a section from a testis of a vitamin A deficient male. This illustration shows the aberrant forms arising from abortive attempts at spermatogenesis.

TABLE 1.—Detailed data on male and female hypophyses (anterior lobe)

Group	Percentage of different cell types			Group	Percentage of different cell types		
	Chromophobe	Alpha	Beta		Chromophobe	Alpha	Beta
Normal females				Normal males			
E813A	52.6	42.5	4.9	N1	46.5	41.6	11.6
E814A	60.4	34.8	4.9	N3	46.4	44.2	9.4
E837	51.1	42.3	6.6	N4	46.4	42.0	11.6
E838	55.4	38.7	5.9	N5	46.8	43.5	9.8
E311	53.2	40.5	6.3	N6	46.1	44.1	9.8
E789	54.7	39.2	6.1	N7	47.0	43.5	9.5
E812	57.9	37.0	5.0	N8	47.8	43.3	8.9
E825	51.4	39.9	8.7	N9	45.8	44.2	10.0
E826	54.4	37.2	8.4	N10	46.1	45.1	8.8
E814R	53.1	39.4	7.5	N11	47.6	43.7	8.7
Mean	54.4	39.1	6.5	Mean	46.7	43.5	9.8
Vitamin A deficient females				Vitamin A deficient males			
E1227	47.4	39.0	13.6	E1228	38.9	36.9	24.2
E1230	44.8	44.0	11.2	E1229	42.5	36.7	20.8
E1231	43.7	42.3	13.9	E1235	40.9	41.6	17.6
E1232	42.4	45.3	12.2	E1257	41.5	37.6	20.9
E1234	43.8	42.3	13.9	E1259	42.9	35.7	21.4
E1236	40.4	43.5	16.0	E1261	47.4	35.2	17.4
E1262	48.8	38.9	12 2	E1268	38.6	37.5	27.9
E1263	51.6	39.2	9.2	E1284	41.9	37.9	20.2
E1267	50.1	39.7	10.2	E1291	41.1	35.4	23.4
E1271	47.7	38.5	13.7	E1313	40.8	38.5	20.6
Mean	46.1	41.3	12.6	Mean	41.6	37.3	21.1
Castrate females				Castrate males			
C-3	40.5	38.1	21.5	C- 9	38.4	39.8	21.7
C-4	41.2	40.7	18.1	C-10	34.6	43.8	21.7
C-5	41.7	40.7	17.6	C-11	35.2	39.1	25.7
C-6	44.3	34.7	21.0	C-12	31.7	43.5	24.8
C-7	43.3	37.8	18.9				
Mean	42.2	38.4	19.3	Mean	35.0	41.5	23.5

Total of cells counted=39,269

TABLE 2.—Summarized data, percentage of different cell types in the anterior lobe of the hypophysis

Group	Cell type (average per cent)		
	Chromophobe	Alpha	Beta
Normal females	54.4	39.1	6.5
Vitamin A deficient females	46.1	41.3	12.6
Castrate females	42.2	38.4	19.4
Normal males	46.7	43.5	9.8
Vitamin A deficient males	41.6	37.3	21.1
Castrate males	35.0	41.5	23.5

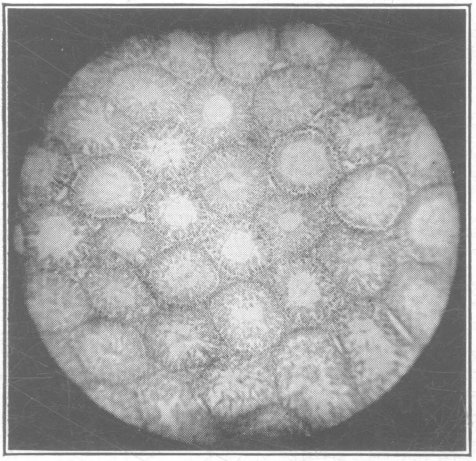

Fig. 1.—Photomicrograph of a normal testis showing normal germinal epithelium and active spermatogenesis

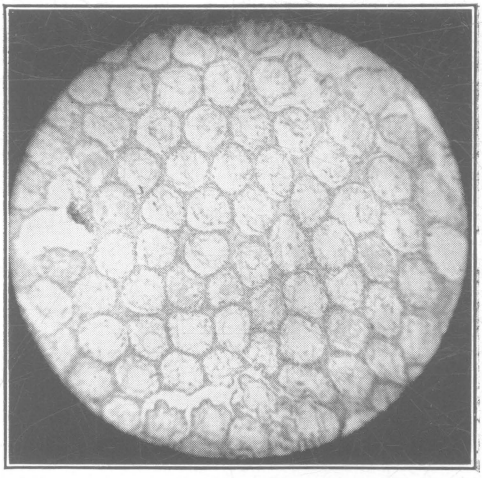

Fig. 2.—Photomicrograph of a testis from a vitamin A deficient animal showing extensive degeneration of the germinal epithelium

The most striking feature of ovarian damage was the absence of large follicles. The medium-sized follicles showed atresia by a sloughing of granulosa cells into the antrum and by an invasion of connective tissue. The injury was not absolute, however, since numerous primary follicles of normal appearance remained. The bulk of the vitamin A deficient rat's ovary was made up of corpora lutea.

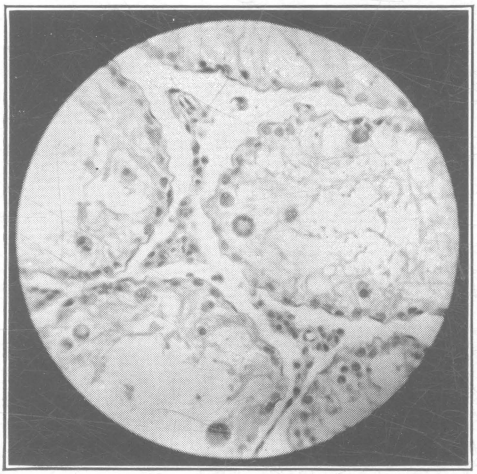

Fig. 3.—Photomicrograph showing the aberrant epithelial forms arising from abortive attempts at spermatogenesis

Histological examination of the vaginae of the deficient animals revealed a cornified epithelium. Occasionally the vaginae appeared to be infiltrated with polymorphonuclear leucocytes. Vaginal smears taken from a group of these animals showed that the leucocyte infiltration occurred at irregular intervals. The uterine glands were frequently found to be cornified, but the lining epithelium in general showed no metaplasia.

PHYSIOLOGICAL CHANGES

Shortly after the hypophysis was shown to be the source of the gonad-stimulating hormone(s), attempts were made to demonstrate that the production of these hormones might be modified. The gonad-stimulating potency of the gland can be demonstrated and assayed by implanting or injecting the gland into immature test animals. The effect of the implanted or injected gland is thus superimposed upon the impotent gland of the test animal, and effective dosages are marked by definite changes in the organs of reproduction. Thus Engle (4) and Evans and Simpson (5) implanted pituitaries from normal and castrate rats into immature rats and mice. They then measured the effects of the implantations by the increase in weight of the ovaries of the test animal. The increase in ovarian weight came from a stimulation of follicular growth. These studies showed that the hypophyses from castrate animals were much

more potent than those from normal animals. Evans and Simpson also showed that the hypophyses from cryptorchid animals evoked a reaction intermediate between that caused by glands from normals and that caused by glands from castrates. Smith, Severinghaus, and Leonard (26) demonstrated that the increased potency of the castrate rabbit hypophyses was associated with an increase in the basophile cells of the hypophysis.

Severinghaus (24) points out, however, that the hormone content of a gland may not be a good measure of its activity. The gland may be merely storing the hormone rather than releasing it into the circulating blood. Thus the increased potency of the hypophysis might actually represent a decrease in activity rather than an actual increase in the production of the hormones.

The increased gonadotropic content of the castrate's hypophysis is not due merely to storage of the hormone, since parabiosis experiments indicate that the active export of the hormone is also increased (18), (12), (31), (2). In these experiments the blood streams of the test animal and the experimental animal are joined and the increase in gonadotropic hormone production in the hypophysis of the experimental animal is reflected in increased activity of the gonads of the test animal. Goto (10) showed the increased hormone content of the castrate's circulating bloodstream by injecting castrate blood into normal females.

To determine whether a relationship exists between the increased number of basophile cells in the hypophysis of the vitamin A deficient animal and the production of gonadotropic hormones, the authors of this work decided to assay the pituitaries. Vitamin A deficient rats are too weak and susceptible to infection to lend themselves readily to the rigors of parabiosis experiments.

The early workers in pituitary assays, as already pointed out, used ovarian weights as a measure of the stimulation of the ovary. The stimulated ovary, however, not only increases in weight but also increases in the production of its hormones. Since there may be a considerable increase in hormone production by the ovarian cells without a marked increase in ovarian cell proliferation, it does not necessarily follow that there is a direct relationship between ovarian hormone output and ovarian weight, especially in the lower ranges of stimulation. The ovarian hormones show their presence by their effects on the uterus and vagina. Levin and Tyndale (14) injected immature mice with gonadotropic substance and measured the ovarian stimulation indirectly by measuring the increase in uterine weights. They were able to show that this method of measurement is three to five times as sensitive for the assay of stimulation as is the ovarian weight method. Heller, Lauson, and Severinghaus (11) used the weight of the immature rat uterus as an assay measurement and confirmed the work of Levin and Tyndale. They make a further statement that this method is simpler and "practically as sensitive as a method based on vaginal histology." Contrary to this last statement, Marrian and Parkes (16) and Szarka and Kurtz (28) state that the amount of hormone required to produce uterine changes in test animals is many times that required to produce vaginal changes.

The present authors used the vaginal changes in the immature white mouse for measuring stimulation.

MATERIALS AND METHODS

After the animal from which the hypophysis was to be analyzed had been deeply anaesthetized it was exsanguinated in order to keep the operative field clear of blood. The top of the skull was removed, the brain scooped out, and the dura mater reflected so that the pituitary gland lay free in the skull.

The pituitary was then transferred to a weighed porcelain crucible and the posterior lobe removed by blunt dissection. It was found that the posterior lobe could be easily freed from the rest of the hypophysis by merely drawing it away with a needle. The crucible and its contents were weighed immediately.

The anterior lobe was then macerated with a glass rod, 1 cc. of distilled water was added to the macerated tissue, and the whole was thoroughly mixed. Distilled water was used as the vehicle in order to rupture, under the influence of osmotic pressure, any cells which might have escaped mechanical disruption. The rupture of the cells frees the contained hormones and precludes the possibility of the cells' "taking" and actively resuming secretion in the test animal. The suspension was then taken up in a tuberculin syringe and a measured quantity injected subcutaneously into the test animal.

The test animals were female white mice, 18 days old at the time of injection. When possible to do so, pituitaries from normal animals and vitamin A deficient animals were injected into individuals in the same litter of mice, to minimize the effect of possible litter variations. One animal was saved from each litter as an uninjected control. Ninety-six hours after injection the mice were killed and ovaries, uterus, and vagina removed for sectioning.

The vaginae from a number of mice were sectioned longitudinally. It was found that the typical reaction was constant over the entire epithelium except near the internal os and near the external opening. Therefore the middle one-third of the vagina was routinely used as the test region, and it was sectioned transversely for the sake of ease of manipulation. The histological changes of the vagina were divided into four stages, modified somewhat from those used by Fluhmann (7) in his oestrin assays. Reaction 1 was that of the uninjected control. The vaginal epithelium was two cell layers thick and the superficial layer was either cuboidal or columnar. Reaction 2 showed stratification with the superficial layer mucified and usually columnar. In reaction 3 the superficial stratum was squamous, and in reaction 4 it was keratinized. Reaction 2 is the earliest reaction attributable to the ovarian hormones, since they are of the nature of growth hormones stimulating cellular proliferation. Typical reactions are illustrated in figures 4, 5, 6, and 7.

Since pituitary weights were recorded, and measured portions of the 1-cc. suspensions injected, it was possible to calculate the weight of pituitary substance injected.

The rats used as pituitary donors were placed on a diet meeting the U. S. P. XI specifications for a vitamin A free diet except that unextracted Argentine casein was used. This diet contained sufficient vitamin A to provide for growth over a period several weeks longer than possible when alcohol-extracted casein is used. Pituitaries from these animals were assayed after mild to moderate symptoms of vitamin A deficiency developed. The normal animals were from the writers' stock colony, and their pituitaries were assayed when the animals were of ages comparable to those of the vitamin A deficient animals.

RESULTS

The results of the assays are presented in tables 3, 4, 5, and 6 and summarized in table 7.

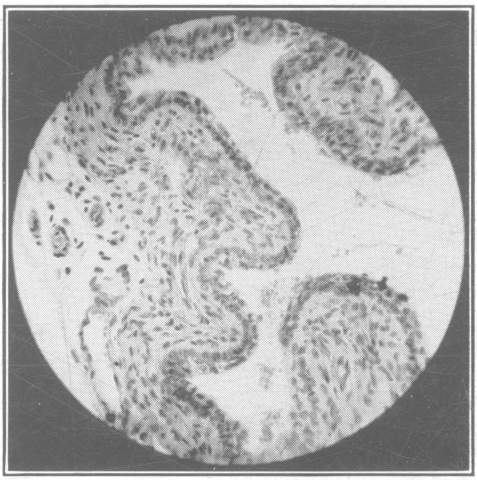

Fig. 4.—The normal vaginal epithelium of a 22-day-old mouse

This is quite typical for the control animals used in this experiment and is designated reaction No. 1.

Fig. 5.—A typical No. 2 reaction

The epithelium consists of more than two layers of cells and the border layer is columnar and mucified.

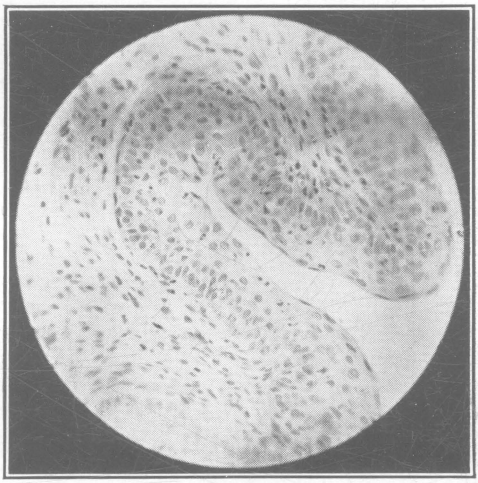

Fig. 6.—A No. 3 reaction

The epithelium is stratified and has a squamous surface layer.

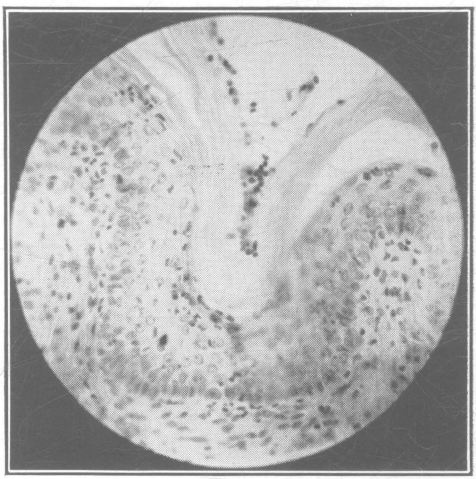

Fig. 7.—The reaction No. 4 is typified by a stratified squamous epithelium with a keratinized surface layer.

TABLE 3.—Assay results obtained when three-tenths of the anterior lobe of rat pituitaries was injected

Pituitaries from normal males				Pituitaries from normal females				Pituitaries from vitamin A deficient females			
Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction	Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction	Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction
	Mg.	*Mg.*			*Mg.*	*Mg.*			*Mg.*	*Mg.*	
59.....	.....	.....	3	257.....	5.1	1.53	2	106.....	3.9	1.17	3
147.....	3.5	1.05	3	259.....	4.4	1.32	3	113.....	5.8	1.74	3
149.....	3.2	.96	3	260.....	3.6	1.08	2	137.....	2.8	.84	3
152.....	3.2	.96	3	261.....	6.2	1.86	3	138.....	3.3	.99	3
154.....	3.8	1.14	3	262.....	4.4	1.32	3	139.....	3.6	1.08	3
155.....	3.9	1.17	3	265.....	5.1	1.53	2	141.....	3.2	.96	3
157..... ..	3.2	.96	3	267.....	3.2	.96	2	145.....	3.5	1.05	3
158.....	2.4	.72	2	269.....	5.0	1.50	2	148.....	3.2	.96	3
159.....	3.0	.90	3	270.....	4.8	1.44	2	150.....	3.0	.90	3
175.....	2.4	.72	2	274.....	3.8	1.14	1	153.....	3.8	1.14	3
Average....	3.17	.95	2.8	Average.	4.56	1.37	2.2	Average.	3.61	1.08	3.0

TABLE 4.—Assay results obtained when two-tenths of the anterior lobe of rat pituitaries was injected

Pituitaries from normal males				Pituitaries from vitamin A deficient males				Pituitaries from normal females				Pituitaries from vitamin A deficient females			
Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction	Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction	Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction	Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction
	Mg.	*Mg.*			*Mg.*	*Mg.*			*Mg.*	*Mg.*			*Mg.*	*Mg.*	
243.....	3.6	0.72	2	208	3.6	0.72	3	263.....	4.9	0.98	1	178.....	2.5	0.50	3
244.....	3.7	.74	3	209.....	2.5	.50	3	268.....	4.1	.82	1	179.....	1.9	.38	3
246.....	3.3	.66	3	211.....	1.9	.38	3	271.....	5.5	1.10	1	181.....	2.1	.42	2
247.....	3.4	.68	3	212.....	2.1	.42	3	273.....	6.3	1.26	1	182.....	3.2	.64	2
248.....	4.1	.82	3	214.....	2.5	.50	3	275.....	3.4	.68	1	184.....	3.7	.74	3
250.....	3.4	.68	3	216.....	2.7	.54	3	309.....	5.7	1.14	1	203.....	3.2	.64	3
252.....	2.7	.54	3	218.....	2.6	.52	3	310.....	6.0	1.20	1	205.....	5.0	1.00	3
253.....	3.9	.78	3	219.....	2.4	.48	3	312.....	4.2	.84	1	206.....	3.1	.62	3
255.....	3.3	.66	3	221.....	3.1	.62	4	313.....	5.7	1.14	2	280	4.4	.88	2
256.....	4.2	.84	1	223.....	2.1	.42	3					281.....	5.0	1.00	3
				224.....	2.3	.46	2								
				225.....	1.9	.38	3								
Average.	3.56	.71	2.7	Average	2.47	.50	3.0	Average	5.09	1.01	1.1	Average	3.41	.68	2.7

TABLE 5.—Assay results obtained when one-tenth of the anterior lobe of rat pituitaries was injected

Pituitaries from normal males				Pituitaries from vitamin A deficient males				Pituitaries from vitamin A deficient females			
Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction	Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction	Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction
	Mg.	*Mg.*			*Mg.*	*Mg.*			*Mg.*	*Mg.*	
276	4.9	0.49	2	227	2.0	0.20	2	282	2.7	0.27	2
303	5.4	.54	3	228	3.6	.36	3	286	5.4	.54	2
304	5.3	.53	3	278	3.4	.34	3	287	3.9	.39	2
305	4.7	.47	3	284	1.7	.17	3	290	2.6	.26	2
307	4.9	.49	2	285	3.1	.31	3	294	2.2	.22	2
308	5.1	.51	2	289	3.6	.36	2	297	2.4	.24	2
314	4.4	.44	2	292	2.6	.26	3	298	4.5	.45	2
316	4.4	.44	2	293	2.8	.28	2	301	2.9	.29	1
317	5.4	.54	2	296	2.3	.23	3	320	2.6	.26	2
319	4.3	.43	2	299	2.9	.29	3	322	2.3	.23	2
								323	1.9	.19	1
Average	4.88	.49	2.3	Average	2.8	.28	2.7	Average	3.03	.30	1.8

TABLE 6.—Assay results when five-hundredths of the anterior lobe of vitamin A deficient male rat pituitaries was injected

Assay mouse number	Total weight of rat pituitary	Weight of pituitary injected	Vaginal reaction
	Mg.	*Mg.*	
325	2.4	0.12	2
326	2.4	.12	1
327	3.0	.15	2
328	3.0	.15	2
329	2.7	.13	2
331	3.0	.15	2
332	2.7	.13	1
334	3.0	.15	2
Average	2.77	.14	1.8

TABLE 7.—Summary of pituitary assays

Donor animals	Amount of anterior pituitary injected		Average vaginal reaction of mouse recipient
	Portion of total gland injected	Average weight of gland injected	
	Pct.	*Mg.*	
10 normal males	30	0.95	2.8
10 normal females	30	1.37	2.2
10 vitamin A deficient females	30	1.08	3.0
10 normal males	20	.71	2.7
12 vitamin A deficient males	20	.50	3.0
9 normal females	20	1.01	1.1
10 vitamin A deficient females	20	.68	2.7
10 normal males	10	.49	2.3
10 vitamin A deficient males	10	.28	2.7
11 vitamin A deficient females	10	.30	1.8
8 vitamin A deficient males	5	.14	1.8
	78 uninjected control mice		1.07

Pituitary injections were also made at levels higher than those presented in these tables. Groups of 10 assay animals each were treated with graded dosages of pituitaries beginning at the level of five-tenths of the total anterior pituitary of the donor animal and progressing downward to levels of four-tenths, three-tenths, etc. At the levels in which more than three-tenths of the total pituitary of the donor animals was injected, the results were somewhat inconsistent. These inconsistencies arose because when these larger dosages were given, the vaginal epithelium itself would frequently present the appearance of a No. 3 reaction whereas the contents of the lumen would indicate that the reaction had passed through the cornified stage (No. 4 reaction). In recording the results, however, the reaction was never given a No. 4 rating unless the cornified layer was attached or lying in close proximity to the surface of the epithelium. Handling the material in this way tended to minimize the differences in potency between the groups injected at the higher levels. These differences in potency became apparent in a striking manner when smaller dosages were given. For instance, a comparison of the data obtained by injecting pituitaries from normal and vitamin A deficient males at the three-tenths level

showed no difference in the average reaction. It was quite evident, however, that in several cases in the vitamin A deficient group the vaginal epithelium of the test mouse had passed through a cornified state as judged by the contents of the lumen. Yet in following the arbitrary decision to consider only the adherent epithelium itself in judging the degree of stimulation, these reactions were given a No. 3 rating.

From their experience with this technique of assaying pituitaries for gonadotropic activity the writers feel that levels of administration which will give average reactions between 2 and 3 are most desirable.

An inspection of the data presented in the accompanying tables reveals some interesting results. The pituitary substance from normal males is more potent than that from normal females. The magnitude of this difference is illustrated by the fact that an average of 0.71 mg. of pituitary substance from normal males gave an average reaction of 2.7, whereas an average of 1.37 mg. from normal females gave an average reaction of 2.2.

The pituitaries of vitamin A deficient animals of either sex were more potent for gonadotropic substance than those from normal animals of the same sex. Pituitary substance from vitamin A deficient males had the greatest potency. An average reaction of 2.7 was obtained from 0.28 mg. of pituitary substance from vitamin A deficient males, whereas it took 0.68 mg. from vitamin A deficient females and 0.71 mg. from normal males to produce the same reaction.

The sensitivity of the assay technique is illustrated by the response obtained when 0.14 mg. of pituitary substance from vitamin A deficient males was injected. This average reaction of 1.8 can hardly be considered insignificant when the average of 78 uninjected control mice was 1.07. That is, only 5 out of 78 mice used as controls in the entire experiment had a vaginal epithelium that could be given a No. 2 rating.

These differences in potency are in agreement with what might be expected from the differences in cellular composition. In other words, those glands containing the higher percentages of basophilic (beta) cells gave reactions showing greater gonadotropic potency.

SUMMARY

A histological study of anterior pituitary glands from normal, vitamin A deficient, and castrate rats revealed a marked increase in the beta cells of glands from vitamin A deficient rats. This change in the cellular elements, although not as great in magnitude as that found in castrate animals, was in the same direction, and might be referred to as a "partial castration effect." The increase in beta cells was more marked in vitamin A deficient males than in vitamin A deficient females. This sex difference coincides with the more extensive degeneration of the germinal epithelium of the male gonad.

A sensitive technique for the assay of gonadotropic activity of pituitary substance was developed. The degree of stimulation was measured by the metaplasia of the vaginal epithelium in the test animal. The sensitivity of this assay technique was illustrated by the fact that 0.14 mg. of fresh anterior pituitary substance from vitamin A deficient male rats gave a measurable response.

Anterior pituitaries from normal and vitamin A deficient rats were assayed for gonadotropic activity by this technique. The data presented show: that pituitary substance from normal males has greater gonadotropic activity than that from normal females; that the gonadotropic activity of pituitary glands increases in vitamin A deficiency; that the increase in potency is greater in male pituitaries than in female pituitaries.

This study provides evidence that vitamin A deficiency exerts a direct damage on the gonads. The anatomical and physiological changes in the pituitary represent compensatory changes in the hypophysis similar to those which follow castration.

LITERATURE CITED

1. Addison, W. H. F. 1917. The cell changes in the hypophysis of the albino rat after castration. Jour. Comp. Neurol. 28: 441.
2. Cutuly, E., D. R. McCullagh, and Eliz. Cutuly. 1937. The type and degree of gonadal stimulation induced in hypophysectomized male rats parabiotically joined with castrated, cryptorchid, and normal partners. Endocrinology 21: 241.
3. Ellison, E. T., and J. M. Wolfe. 1934. The effect of castration on the anterior hypophysis of the female rat. Endocrinology 18: 555.
4. Engle, E. T. 1929. The effect of daily transplants of the anterior lobe from gonadectomized rats on immature test animals. Amer. Jour. Physiol. 88: 101.
5. Evans, H. M., and M. E. Simpson. 1929. A comparison of anterior hypophyseal implants from normal and gonadectomized animals with reference to their capacity to stimulate the immature ovary. Amer. Jour. Physiol. 89: 371.
6. ———— and ————. 1930. Subnormal sex-hormone content of the hypophysis of animals with inadequate antineuritic vitamin B. Anat. Rec. 45: 216. Abstract of paper presented at 46th annual session of the American Association of Anatomists.
7. Fluhmann, C. F. 1934. A new procedure for the demonstration of estrin in the blood of women. Endocrinology 18: 705.
8. Foot, N. C. 1933. The Masson trichrome staining methods in routine laboratory use. Stain Tech. 8: 101.
9. Frank, M. 1937. Untersuchen über experimentelle Beeinflussung der Vitamine A Mangelkrankheit. Arch. f. Kinderheilk. 110: 91. Abstracted in Nutr. Abst. and Rev. 7: 39 (1937).
10. Goto, N. 1925. Experimentelle Untersuchung der inneren Sekretion des Ovariums durch Rattenparabiose. Arch. Gyn. 123: 377.
11. Heller, C. G., H. Lauson, and E. L. Severinghaus. 1938. The immature rat uterus as an assay end-point for gonadotropic substances. Amer. Jour. Physiol. 121: 364.
12. Kallas, H. 1929. Parabiose und Hypophysenvorderlappen. Arch. f. d. Gesch. Physiol. 223: 232.
13. Korenchevsky, V. 1923. Glands of internal secretion in experimental avian beri-beri. Jour. Path. and Bact. 26: 382.
14. Levin, L., and H. H. Tyndale. 1937. The quantitative assay of "Follicle-Stimulating" substances. Endocrinology 21: 619.
15. Marrian, G. F., and A. S. Parkes. 1929. The effect of anterior pituitary preparations administered during dietary anoestrus. Proc. Roy. Soc. London, Series B, 105: 248.
16. ———— and ————. 1930. The relative amounts of oestrin required to produce the various phenomena of oestrus. Amer. Jour. Physiol. 69: 372.
17. Mason, K. E., and J. M. Wolfe. 1930. The physiological activity of the hypophyses of rats under various experimental conditions. Anat. Rec. 45: 232. Abstract of paper presented at the 46th annual session of the American Association of Anatomists.

18. Matsuyama, R. 1921. Experimentelle Untersuchungen mit Rattenparabiosen. III. Die Veränderungen der Geschlectsdrüsen und der Organe, die damit in inniger Beziehung stehen. Frankf. Zschr. Path. 25: 436.
19. Mitzkewitsch, M. S. 1934. Der Zustand dur endokrinen Drüsen während der A—Avitaminose bei Ratten. Arch. f. Exper. Pathol. u. Pharmakol. 174: 339.
20. Nelson, W. O. 1933. Studies on the anterior hypophysis. 3. The anterior hypophysis in vitamine E-deficient rats. Anat. Rec. 56: 241.
21. Nukariya, S. 1926. Über die Bedeutung der Rückresorption des Spermas (auf Grund von Spermainjektion an Kastraten) und über mikroskopische Veränderungen der Hypophyse an jungkastrierten weiszen Ratten. Arch. f. d. Gesch. Physiol. 214: 697.
22. Orten, A. U., and A. H. Smith. 1937. The effect of dietary protein on endocrine function and on blood picture of female rats. Amer. Jour. Physiol. 119: 381. Abstract of paper presented at the 49th annual meeting of the American Physiological Society.
23. Parkes, A. S. 1928. The nature of the anoestrus condition resulting from vitamin B deficiency. Quart. Jour. Exper. Physiol. 18: 397.
24. Severinghaus, A. E. 1937. Cellular changes in the anterior hypophysis with special reference to its secretory activities. Physiol. Rev. 17: 556.
25. Smith, P. E. 1926. Hastening development of female genital system by daily homeoplastic pituitary transplants. Proc. Soc. Exper. Biol. Med. 24: 131.
26. ————, A. E. Severinghaus, and S. L. Leonard. 1933. The effect of castration upon the sex-stimulating potency and the structure of the anterior pituitary in rabbits. Anat. Rec. 57: 177.
27. Stein, S. I. 1935. Experimental studies on the hypophysis cerebri. IV. The effect of vitamin E deficiency in the female albino rat. Jour. Nutr. 9: 611.
28. Szarka, A. J., and G. Kurtz. 1938. A study of the ratio of the amount of theelin producing uterine and vaginal estrus. Endocrinology 23: 64.
29. Thatcher, H. S., and B. Sure. 1932. Avitaminosis. III. Pathologic changes in tissues of the albino rat during early stages of vitamin A deficiency. Arch. Path. 13: 757.
30. Van Wagenen, G. 1925. Histological changes in the male rat hypophysis following degeneration of the germinal epithelium. Anat. Rec. 29: 398. Abstract of paper presented at the 41st annual session of the American Association of Anatomists.
31. Witschi, E., and W. T. Levine. 1934. Oestrus in hypophysectomized rats parabiotically connected with castrates. Proc. Soc. Exper. Biol. Med. 32: 101.
32. Wolbach, S. B., and P. R. Howe. 1925. Tissue changes following deprivation of fat-soluble A vitamin. Jour. Exper. Med. 42: 753.
33. Wolfe, J. M., and R. Cleveland. 1933. Cyclic histological variations in the anterior hypophysis of the albino rat. Anat. Rec. 55: 233.

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